Flowering, pollination, and fruit set in Tongue Orchids *Cryptostylis* spp.

AC Gaskett and ME Herberstein

Department of Biological Sciences, Macquarie University, NSW 2109
Corresponding author: agaskett@bio.mq.edu.au

Abstract

Study of Australian Tongue Orchids addresses questions of widespread interest about the evolution of sexually deceptive pollination, and provides information for conservation and management. We present recent data on flowering, pollination, and fruit set for three *Cryptostylis* species: the Bonnet Orchid *C. erecta* RBr, the Small Tongue Orchid *C. leptochila* F Muell. *Ex* Benth. and the Large Tongue Orchid *C. subulata* (Labill.) HG Reichb. (Jones 1988). These species are pollinated by male Orchid Dupe Wasps *Lissopimpla excelsa* (Ichneumonidae) when they 'pseudocopulate' with the flowers. *Cryptostylis subulata* flowered from December to February, and *C. erecta* flowered from November to March. *Cryptostylis leptochila* began flowering in December, and pollination was still occurring in late April. This species had the most flowers, but the lowest fruit set. In most field sites, the earliest flowers on a raceme were pollinated most often, although this did not occur when pollinators were scarce. Orchids may attract pollinators more easily at the start of the flowering season before the female wasps emerge, or pollinators could learn the locations or appearance of orchids and avoid later-opening flowers. We also found that pollinator abundance varied during and between seasons, there was no evidence of self-pollination, and *C. erecta* racemes were more likely to be eaten by predators after fruit set. (*The Victorian Naturalist* **123** (3), 2006,128-133)

Introduction

Species from the fascinating terrestrial orchid genus *Cryptostylis* are distributed throughout Australasia and the South Pacific (Jones 1988). There are five Australian species: the Bonnet Orchid *Cryptostylis erecta* RBr, Small Tongue Orchid *C. leptochila* F Muell. *Ex* Benth., Large Tongue Orchid *C. subulata* (Labill.) HG Reichb., Leafless Tongue Orchid *C. hunteriana* Nicholls and Slipper Orchid *C. ovata* RBr (Jones 1988).

The abundance and rarity of *Cryptostylis* species vary throughout their distributions. For example, *C. erecta* is common in NSW (Bishop 2000), but is listed as 'vulnerable' under Victorian legislation (Flora and Fauna Guarantee Act 1988). *Cryptostylis leptochila* can be locally common in Victoria and New South Wales, but is listed as 'endangered' in Tasmania's Threatened Species Protection Act 1995. One species, *C. hunteriana*, is extremely rare throughout its range in Victoria, New South Wales, and Queensland (Bell 2001; Clark *et al.* 2004). It is considered 'threatened' under the Victorian *Flora and Fauna Guarantee Act 1988* and 'vulnerable' under both the NSW *Threatened Species Conservation Act 1995* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999.* Research into the natural history of these species is valuable for the preparation of recovery plans, and general conservation and management activities. Furthermore, study of the most common *Cryptostylis* species in their areas of greatest abundance provides information that may be applied to rare *Cryptostylis* species, and other orchids with similar sexually deceptive pollination systems.

Whilst *C. hunteriana* is a leafless saprophyte, all other Australian *Cryptostylis* species have a solitary, evergreen leaf (Jones 1988). In *C. erecta* and *C. leptochila*, the leaf underside is purple. The flowers of *Cryptostylis* are resupinate with a very large labellum that is predominantly red or burgundy (Jones 1988). Plants can produce a single flower raceme between August and April. The multiple inflorescences on the raceme are thought to open sequentially throughout the flowering season (Jones 1988). The frequency of flowering in individual plants appears to vary unpredictably between years, a common characteristic of terrestrial orchids (for a review, see Kindlemann and Balounová 2001).

Cryptostylis species attract pollinators by sexual deception. The orchid flowers are thought to mimic the appearance and scent of female insects. Male insects that respond to the mimicry and attempt to copulate with the orchids' flowers inadvertently collect and distribute the pollinia. Australian *Cryptostylis* species are pollinated by males of a single species of Ichneumonid wasp, the Orchid Dupe *Lissopimpla excelsa* (Costa) (CSIRO 1991). For first-hand descriptions of pollination in *Cryptostylis* species see Coleman (1928, 1929, 1930), Dacy (1974), Watson (1961), and Stoutamire (1974). Although *Cryptostylis* species share a pollinator, and often have overlapping flowering seasons and distributions, no hybrids have been reported between species (Stoutamire 1974; Jones 1988). Cross-pollination of the species by hand suggests there are strong internal mechanisms that prevent hybridisation (Stoutamire 1974; Jones 1988; Lloyd 2003).

Pollinators are initially attracted to *Cryptostylis* orchids with a chemical signal thought to mimic sex pheromones emitted by female *L. excelsa* wasps (Schiestl *et al.* 2004). Other visual and tactile signals, e.g. colours, shapes, and textures that resemble the features of female wasps, may then stimulate males to attempt to copulate with the flower, and thus move vigorously enough to transfer pollinia. Deception by orchid flowers may impose costs upon duped insects (e.g. Wong and Schiestl 2002), and insect behaviour and learning may influence pollination success (e.g. Ferdy *et al.* 1998).

Here we report some interesting recent field observations and data on flowering, pollination, and fruit set for three species of *Cryptostylis*: *C. erecta*, *C. leptochila*, and *C. subulata*.

Methods

Field observations were made of natural populations of *Cryptostylis erecta*, *C. subulata*, and *C. leptochila* in open woodlands in New South Wales and Victoria. We used two populations of sympatric *C. erecta* and *C. subulata* near Sydney and Nowra, and one sympatric *C. leptochila* and *C. subulata* site near Melbourne. The fourth site, near Nowra, had only *C. subulata*. At each site we identified patches of orchids for study. A patch was defined as a cluster of plants that was more than two metres from any other *Cryptostylis* plants (Table 1).

In the summer of 2003-04, we visited the Sydney *C. erecta* and *C. subulata* site eight times throughout the flowering period and made detailed observations of individually labelled plants. We recorded the period for which individual flowers were open, the interval until pollinia collection and/or deposition, and the occurrence of fruiting, seed set, and predation.

During summer 2004-05, we visited all four field sites three times and made less intensive observations of flowering, seed set, and predation. Patches of orchids measured at the Sydney site during the first field season were not remeasured during the second year of the study. Analyses were pooled for each species, and confidence intervals of 95% were used.

At each field site, regression analyses were used to determine whether the position of a flower along a raceme (i.e. how early in the season it opened) affected its likelihood of being pollinated. For these analyses, the dependent variable was the proportion of pollination that occurred for flowers in each position along a raceme. The data were pooled according to field site because all *Cryptostylis* species share a single pollinator and pollinator abundances

Table 1. Number of patches surveyed for three species of Tongue Orchid *Cryptostylis* at four sites in New South Wales and Victoria. *denotes data combined from two study seasons, Summer 2003-04 and Summer 2004-05.

site	*C. erecta*	*C. leptochila*	*C. subulata*	*C. erecta* and *C. subulata*
Sydney	26*	-	5*	1
Nowra 1	1	-	6	1
Nowra 2	-	-	7	-
Melbourne	-	15	8	-
Total	27	15	26	2

may differ between the field sites. In two final regression analyses on the effect of flower position, we used data from *C. erecta* at the Sydney site for each of the two study seasons to compare pollination between years.

To test for self-pollination, we selected four pairs of flowering *C. erecta* plants in the Sydney field site. We isolated each plant in a mesh bag that prevented insect access to the flowers. One plant of each pair was hand-pollinated during the season. The second plant was not hand-pollinated, but used as a control. All the racemes were checked for fruit set during and after the flowering season.

We also assessed pollinator abundance and activity in an ad hoc manner by considering the time necessary to capture wasps on different days throughout the 2004-05 flowering season at the Sydney field site. Wasps were captured with a hand net when they arrived at our 'bait' flowers, as described by Peakall and Handel (1993) and Bower (1996).

Results and Discussion

Data were collected from 70 patches of orchids, including two patches of mixed *C. erecta* and *C. subulata*, which were excluded from subsequent analyses about single species patches. See Table 1.

Flowering seasons

In all sites, *C. subulata* had the shortest flowering period of the three species (December to February). For *C. erecta*, flowering commenced in November and had mostly finished by early March, although one plant with a flower was found in a sheltered area near a creek in May 2005. The populations of *C. leptochila* near Melbourne had a very long flowering season that began in December and finished as late as April, consistent with Backhouse and Jeanes (1995). Others have reported the flowering season for *C. leptochila* to end in February (Clyne 1970; Jones 1988), or March (Bishop 2000). In March, 48 racemes (92%) still had open flowers, but by May, only three racemes were still active (5.8%). Successful pollination occurred as late as April (nine flowers on seven different racemes).

Plant density and flowering

The average number of plants in each patch was highest in *C. erecta*, and lowest in *C. subulata*, but one patch of *C. subulata* had 900 plants (Table 2). The number of racemes per patch was similar for all three species, but *C. leptochila* had a higher average number of flowers per raceme (Table 2). One plant of *C. leptochila* had 35 flowers, which is three times the maximum number of flowers reported by Jones (1988), and twice that reported by Bishop (2000).

After opening, the flowers of *C. erecta* and *C. subulata* had pollinia collected or deposited after an average of 3.1 days. Some flowers were visited on the day they opened, and the maximum time until pollination was 8 days, but this was for a flower with a damaged labellum. On average, each flower was open for 6 days (min. = 1, max. = 9). Generally, each flower opened as the previous flower on the raceme was closing. Sometimes a flower opened up to six days before the previous flower closed. However, in one case, nine

Table 2. Flowering, pollination, and fruit set in three species of Tongue Orchids *Cryptostylis*. Values with parentheses are: mean (min., max.).

	C. erecta		*C. leptochila*	*C. subulata*	
	2003-04	2004-05	2004-05	2003-05	2004-05
plants surveyed	696	806	754	271	1687
% plants in flower	14.4	4	9.3	5.9	3.4
racemes per patch	8.3 (1, 27)	4 (1, 16)	4.7 (1, 10)	5.3 (1, 8)	2.5 (1,10)
flowers per raceme	5.5 (3, 12)	5 (3, 11)	9.6 (2, 35)	7 (3, 12)	4.7 (1, 11)
pollinated flowers per raceme	2.7 (0, 9)	1.13 (0, 7)	0.28 (0, 3)	2.86 (0,9)	0.77 (0, 5)
% plants that set fruit	72.6	71.9	27.6	75	50
% plants without fruit	19.4	27.1	54	25	38
% racemes predated	8	1	18.4	0	12

days passed between the closing of one flower and the opening of the next on the same raceme.

Pollination and fruit set

Despite the large number of flowers per raceme produced by *C. leptochila*, this species had the lowest average number of pollinated flowers per raceme and the lowest percentage of plants with some fruit set (Table 2). Approximately 70% of *C. erecta* and *C. subulata* plants had at least one pollinated flower in 2003-04, but only 50% of *C. subulata* were pollinated in the 2004-05 season. Schiestl *et al.* (2004) reported pollination rates of 85% for *C. erecta* and *C. subulata* in the Blue Mountains near Sydney in 2000. These data demonstrate that *Cryptostylis* species have a higher rate of pollination than that typically expected for orchids with deceptive pollination syndromes in the temperate southern hemisphere (~40%: Neiland and Wilcock 1998) and globally (~20%: Tremblay *et al.* 2005).

For three of the four field sites, the position of a flower along a raceme significantly affected the likelihood of pollination (Sydney: $R^2=0.53$, $F_{1,15}=15.52$, $p<0.05$; Nowra 1: $R^2=0.45$, $F_{1,11}=8.03$, $p<0.05$; Nowra 2: $R^2=0.16$, $F_{1,7}=1.37$, $p>0.05$; Melbourne: $R^2=0.53$, $F_{1,33}=33.96$, $p<0.05$). Pollination was most likely for flowers that opened earlier in the season (Fig. 1). Coleman (1928) suggested this was because the male *Lissopimpla excelsa* emerged earlier than females, and were most active as pollinators until the females were available. A second explanation may involve the learning abilities of the male wasps. In several sexually deceptive pollination systems, pollinators initially are

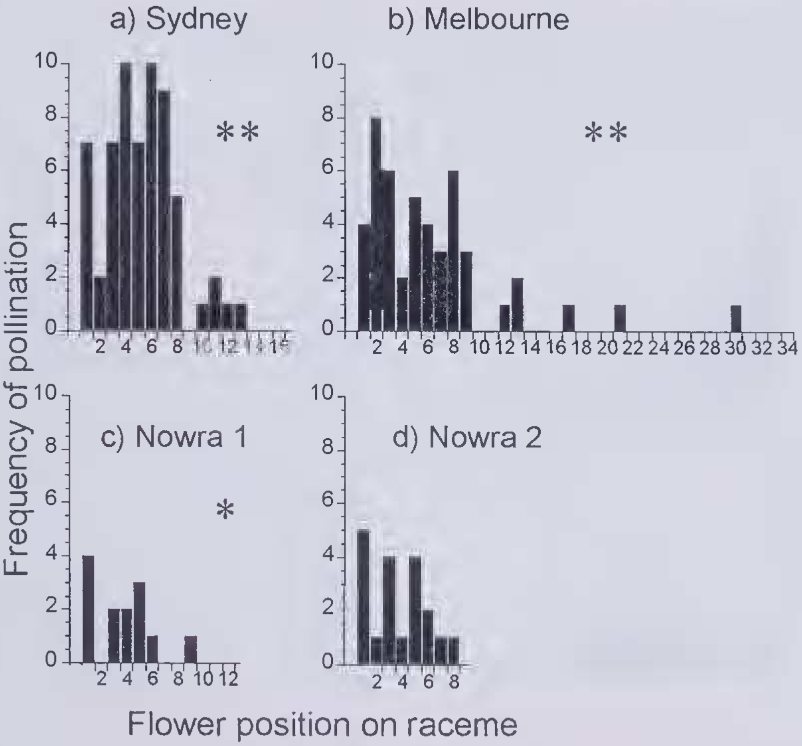

Fig. 1. Frequency of pollination of each sequentially-opening flower positioned along racemes of Tongue Orchids *Cryptostylis erecta*, *C. leptochila*, and *C. subulata*, in four sites in open forest in New South Wales and Victoria. Maximum value on x-axis is maximum number of flowers per raceme at site. Statistically significant effect of flower position at <0.005 level denoted by **, at < 0.05 level denoted by *

attracted strongly to a flower, but this decreases rapidly over a short period, presumably as the duped male pollinators learn that the flower is not a real female insect (e.g. Peakall *et al.* 1990; Peakall and Handel 1993; Wong and Schiestl 2002). Male wasps may remember and avoid the location of a false signal for some time, thus subsequent flowers on a raceme may not be visited. Furthermore, male ability to recognise flowers as false signallers may be frequency dependent and increase with repeated exposure (Ferdy *et al.* 1998).

The impact of male insect learning on orchids' pollination success also may depend upon pollinator abundance. In 2003-04, the effect of flower position on fruit set in Sydney *C. erecta* was highly significant (R^2=0.745, $F_{1,11}$=29.15, $p<0.001$), and the first flower to open on any raceme had a very high frequency of pollination (Fig. 2). However, during the second study season at this site, there were fewer pollinator visits, flowers 1-8 on racemes of *C. erecta* had similar pollination frequencies (Fig. 2), and flower position had no significant effect on pollination (R^2=0.195, $F_{1,12}$=2.671, $p>0.05$). As more flowers open during the season, males' frequent exposure and subsequent learning may lead to avoidance of most flowers.

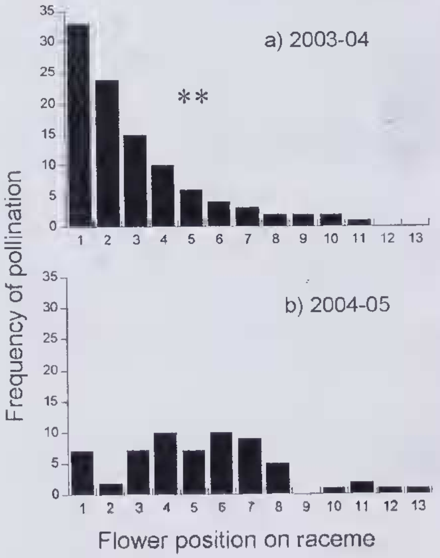

Fig. 2. Frequency of pollination of each sequentially opening flower positioned along racemes of the Bonnet Orchid *Cryptostylis erecta* in an open forest site near Sydney during two flowering seasons: a) 2003-04 and b) 2004-05

Pollinators appeared most active between approximately 9.30 am and 2 pm on warm, sunny days. There were obvious peaks in abundance on certain days in different regions. For example, on one day during February 2005, nine wasps were caught in less than two hours at the Sydney field site (~4.5 wasps/hour). Previous capture efforts in the same area during January and February resulted in only four wasps in 18.5 hours searching over eight days (~0.2 wasps/hour).

Only two of the four pairs of bagged and isolated inflorescences survived the season. However, only those flowers cross pollinated by hand set fruit. None of the flowers in the control bags set fruit. This low sample size still corroborates evidence provided by Dacy (1974), Jones (1988), and Lloyd (2003).

Predation

The predation of racemes was quite low for all species except *C. leptochila* (Table 2). The higher level of predation at the Melbourne field site may have contributed to the lower pollination success for this species. Data from 2003-04 showed that 87.5% (n = 8) of the *C. erecta* racemes that were eaten by predators had recently set fruit. The fleshy fruit of *Cryptostylis* seem to be attractive food for browsing animals.

Despite their shared pollinator, and similar habitat and flowering season, the *Cryptostylis* species varied considerably in their patch sizes, flower numbers, fruit set, and predation rates. *Cryptstylis leptochila* appears to invest heavily in flowering, producing many flowers during an extended season. These features have been associated with strategies to maximise pollination success in other deceptive orchids (Neiland and Wilcock 1995; Kindlemann and Balounová 2001; Tremblay *et al.* 2005). However, *C. leptochila* had the lowest fruit set of the species studied. This may mean that extra investment in flowering has little effect on fruit set, particularly if there are other negative impacts, e.g. predation of fruits.

The generally high fruit set we observed suggests that, unlike many other orchid species, pollinator limitation is not a major conservation issue for *Cryptostylis* species. Management strategies could prioritise protecting plants from predation during the flowering season and conserving suitable open forest habitat (see Clark *et al*. 2004). In addition, care should be taken if information about *Cryptostylis* species is used to develop conservation plans for other orchid genera, which are likely to have considerably lower pollination rates (see Tremblay *et al*. 2005).

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